den norske Mor & barn undersøkelsen

PROTOCOL

The Norwegian Mother and Child Cohort Study

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Norwegian Institute of Public Health

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PROJECT DESCRIPTION

The Norwegian Mother and Child Cohort Study

Summary

In order to achieve better health for mothers and children in the future, we wish to test specific hypotheses about the causes of a number of serious diseases by recruiting 100,000 pregnant women to a cohort study. Possible causal factors will be linked to information obtained from questionnaires, blood samples from mother, father and child, urine sample from mother and medical registries. The Norwegian Mother and Child Cohort Study has multiple endpoints. Primarily those associated with adverse pregnancy outcomes will be studied, as well as diseases affecting mother, father or child. Endpoints will be taken from questionnaires and medical registries. The study will be carried out nationally and researchers with relevant questions will be welcome to participate. No interventions will be undertaken, which means that any conditions that may, potentially, expose the families to disease will not be modified. Both basic and applied research will be undertaken, with projects spanning from molecular genetics to welfare. The Cohort study is now nationwide.

Background

The background for the project is lack of understanding about the causes of disease. The main aim is prevention. This can be achieved by identifying the environmental factors that are the links in the causal mechanism leading to disease. The research has to be specific, and planned in a way that allows concrete questions to be answered. The prevalence of a disease, and the number of causal factors involved, must be taken into consideration. Many people are affected by the serious illnesses with which we are concerned, sometimes at a very young age. All of these diseases are the result of a chain of causal events with many components. There is no contradiction between our understanding of disease as multifactorial (or that health is a multidimensional concept) and looking for specific causes for disease. Much can be gained by understanding the critical points in the causal chain, as experience from the diseases we have been successful in preventing and treating has shown.

The causes of the many diseases and complications that can arise during pregnancy are largely unknown, for example still births or serious congenital abnormalities. We know little about why some births occur prematurely. For many diseases that occur throughout childhood, such as diabetes, autism, cancer, rheumatism and allergy, our knowledge is very incomplete, and treatment of these childhood diseases requires large resources. Many of the complaints and illnesses that occur during pregnancy are also poorly understood. These include nausea, pre-eclampsia, pelvic pain and depression.

Knowledge about the causes of disease (epidemiology) is important for several reasons. Finding specific causes can lead directly to prevention. If we know that a toxic substance or medicament causes damage to a fetus, then by avoiding contact with these substances, we can prevent damage. Similarly, if the damage is caused by an infection, we can give advice in order to prevent infection and develop vaccines. Also, if we know more about the causes of disease here will be less unnecessary anxiety. We have a tendency to blame either ourselves

or factors in our lifestyle or diet, when we are ignorant about the causes of disease. These claims can be tested in the Mother and Child Cohort Study.

Another reason for carrying out epidemiological research is to aid in the development of new medicines. Including mother, father and child in the cohort study will enable us to make effective use of new methods in genetic epidemiology. The transmission disequilibrium test can be used to identify the genes associated with disease. In turn, detailed laboratory work can be carried out which identifies the fundamental metabolic errors underlying disease. Medicines can, therefore, be targeted more efficiently. From this perspective, the cohort study will stimulate basic research in molecular genetics both in Norway and internationally.

It is also important to examine commonly held beliefs concerning the causes of disease. Many aspects of modern society can be sources of anxiety. One recent example is the question as to whether mobile telephones can lead to illness. From a biological point of view this seems unlikely, but without scientific evidence this cannot be refuted. Reduction of unwarranted anxiety is a valuable aspect of the project.

Yet another dimension of the project is to examine quality of life and positive aspects of health. The project can illuminate which environmental factors promote absence of illness and healthy living.

Norway has a social infrastructure that facilitates epidemiological research. We can track individuals and generations over long periods of time. The population is well educated and Norwegians have a long tradition of voluntary participation in responsibly carried-out medical research. In addition, several health registries with high quality data already exist.

The cohort study will be conducted over an extended period of time. Children will be followed up until they are adults, and parents will be followed up over many years. This will enable an investigation into serious adult diseases, such as cancer and cardiovascular disease. For example, all women can be monitored for breast cancer: an illness that is increasing in Norway, especially among the under fifty's. The data collected will enable the testing of many important causal hypotheses.

The project will also supply valuable information relating to the causes of long absences from work during pregnancy. Within the research that is being performed on the Norwegian welfare state system, there has been a lack of individual follow-up studies that include health variables. Which factors are most important in predicting long-term disability? Many women experience that illnesses such as pelvic pain, which start during pregnancy, can affect their subsequent health and ability to return to work.

We believe that if we can contribute to the understanding of even a few of the diseases that we will be investigating, the effort will be worthwhile. The knowledge that is derived will be applicable both to future generations and for mothers and children outside Norway. From a strict philosophy of science viewpoint, we acknowledge that it is impossible to demonstrate that a particular exposure is a cause. It is still correct to use causality models (rather than more diffuse risk models) in order to make the aims of the study more precise and easy to grasp.

Study aims

The study aims to calculate the degree of association between potential causal factors (exposures) and ill health in mother and child.

As the project evolves, new causal hypotheses will arise. Laboratory techniques in the years to come will ensure that improved methods are available for tracing exposures in the biological material. The main data set from the questionnaires, urine- and blood samples will be linked to information about many diseases through other databases. In this way, the main project can be seen to be fundamental to all the subprojects. Many research questions can be answered using the main data set alone, while others will require additional data. If a subproject requires further contact with the study participants in order to collect more data, new consent will be requested.

In addition, the distribution of exposures in the population will be described, and estimates of the incidence and prevalence of many diseases will be made. In the case of some diseases and conditions, natural progression can also be described. No interventions will be carried out as part of the main project.

A number of research questions and proposals for subprojects have been outlined. The breadth of interest in the project is apparent. The many projects focusing on women's health and working conditions during pregnancy will give results relatively quickly. Others, such as those focusing on childhood illnesses, will require follow-up over many years.

Diet, infections, hereditary factors, environmental toxins, medication and exposure to occupational hazards can be mentioned as examples of exposures that are of scientific interest. However, they will not be detailed in this protocol.

Design

This is a cohort study, which involves recruitment prior to the onset of the disease that is to be studied. The aim is to compare the incidence of disease in a group of exposed women/children with a group that has not been exposed, while controlling for other factors which can affect the risk. The main project will provide data regarding disease and exposure variables, which can be analyzed without further modification. Links to health registries (e.g. Medical Birth Registry or Cancer Registry) or exposure registries (National census data) will enable the generation of new data sets, simply and effectively.

Many of the subprojects will be based on the cohort design of the main project. When additional exposure data are required, or blood- and urine samples are being analyzed, the relevant design will be a nested case-control study. This involves identifying a sample of subjects that have developed the disease to be studied, and selecting controls that have not developed the disease, and measuring exposure for both groups.

In addition, subcohorts with particular exposures can be followed in more detail. Also, estimates of the joint effects of genes and disease can be made with the mother-father-child triad design.

Sample

The target population is 100 000 pregnant women who will be recruited between 1999 and 2007. The intention is to continue the project until the goal of 100 000 participants is reached. In Norway, there are approximately 60 000 births annually. We aim to recruit women from every county.

A sample size of 100 000 is required because many diseases are relatively rare and exposures (e.g. certain infections) infrequent. A typical congenital malformation has a prevalence at birth of about 1 per 1000, so 100 cases can be expected. But such malformations are clinically heterogeneous, so in practice, it will be desirable to carry out analyses in subgroups. For some malformations, and for other rare conditions such as childhood cancers, collaboration with the Danish Mother and Child Cohort Study will therefore be indicated.

Four conditions can be seen to lead to a potential selection bias. Firstly, women who choose to terminate pregnancy prior to recruitment to the study or who have a spontaneous abortion will obviously be excluded from the study. Secondly, some women may not wish to participate in the study; thirdly women may withdraw from the study after recruitment and fourthly, women may leave the study because of natural causes such as death or emigration. These selection mechanisms will be described, so that it is possible to examine their likely effect on the study aims. The most important factor affecting selection is probably low recruitment. When calculating associations between exposure and disease this can be critical, but not necessarily. However, low recruitment will commonly bias estimates of disease prevalence, particularly if there is systematic variation according to parents' education or other factors that may be associated with willingness to participate.

Case-control sampling will be based on the number of cases that are found. Normally, two to four times as many control cases are required. These will be randomly selected from the whole cohort. A control group can be used for different groups of cases.

Variables

Exposure variables

An exposure variable is defined as one if it is named in the study aims as a potential causal factor. Many of the questions posed in the questionnaire will either directly or indirectly measure exposure. Similarly, specific blood- and urine factors will be exposure variables when they are analyzed as causal factors. A fuller understanding of the framework for these variables can be obtained from examining the content of the questionnaires (appendix 7-10) and the detailed description of blood- and urine sample collection and storage (appendix 2). Subprojects will also enable the collection of further exposure variables.

Health Variables

A health variable is defined as one that describes or defines a health condition, either from the questionnaire, registry or urine- and blood samples. Normally these variables become effect variables in a cause-effect model, but they can also be exposure variables for other health variables. For example a mother's mental health can be the cause of a child's future psychiatric complaint, and low birth weight can lead to a number of childhood ailments. Many health variables will be collected from links to other health registries or as part of subprojects.

Other variables

The questionnaire will also include a number of other variables which conceptually are neither exposure nor health variables. Some of these will correct for known associations, while others are included because they are of general research interest. Many of the questions relating to diet and use of medicaments are of this type.

Table 1
Examples of exposures and health outcomes to be investigated in the Norwegian Mother and Child Cohort Study.

Examples of exposures	**Examples of diseases**
Medication	Pelvic pain
Hereditary factors	Congenital malformations
Infections	Stillbirth
Dietary factors	Premature birth
Environmental toxins	Cancer
Physical activity	Diabetes
Work situation	Asthma/allergy
Occupational hazards	Rheumatism
Interpersonal relationships	Depression
Personal habits	Breast cancer

Data collection

Recruitment
An invitation for participation in the study is sent to women at their home address. The majority will receive this package three weeks before attending routine ultrasound examination in the 17.-19.th week of pregnancy. Names and addresses are obtained from the clinics that have received a request for ultrasound examination, either from a doctor or the woman herself. Each week the ultrasound clinics send a list of all women who have appointments to the Medical Birth Registry (MBR). This list includes names, addresses and national identity number, as well as the date of the appointment (see appendix 1). Permission to maintain such a list of names has been granted by the Norwegian Data Inspectorate. This list is used by the clinics to prepare labels, expected number of blood- and urine samples and also as a means of calculating the rate of non-participation.

The invitation, which is sent out in collaboration with each participating hospital, describes the purpose of the study, protection of privacy and practical details. It is emphasized that participation is voluntary, and that consent from children will be sought when they are older. The women are also notified that they can withdraw at any time. In addition, women are informed that additional invitations may be received requesting participation in subprojects. This may entail the collection of further data. Information brochures about the project together with Questionnaires I are enclosed. Also enclosed is a consent form, which requires a signature, and a return-paid envelope.

If a woman wishes to participate, the questionnaires and signed consent form are returned either by post to the Medical Birth Registry in Bergen, or handed-in at the ultrasound examination.

Ultrasound examination
At this examination, a midwife informs about the project and asks the woman if she wishes to participate. After obtaining consent, urine- and blood samples are drawn. Blood samples are also taken from the participating fathers. Blood- and urine samples are sent the Norwegian Institute of Public Health in Oslo. See appendix 2. If the mother and father are uncertain about participation, they can either consult the midwife or she may refer the couple to a project colleague who can clarify any misunderstandings. Two weeks later all women are sent news from the study as a single reminder.

A copy of the standardized ultrasound form where values can be entered and any abnormal findings noted is sent from the hospital to the Medical Birth Registry.

Prenatal questionnaires
During the 22th and 30th week, the woman is sent new questionnaires (Questionnaire II and III) with a reply paid envelope. If necessary, a reminder is sent after three weeks.

At Birth
Soon after birth, a blood sample from the umbilical cord and a second sample from the mother are taken. Both are sent to the Norwegian Institute of Public Health in Oslo.

Postnatal questionnaires
Further questionnaires are sent to the mother when the child is six months, eighteen months, three years and six years old.

Data storage

Person identifiable data
Women who agree to participate are registered in a data base containing name, national identification number, a code number which corresponds to other files, information relating to when questionnaires or blood- and urine samples are sent and received, and whether reminders have been sent. No other information is stored in this database. All information about father and child will be linked to the woman, who is the index person in the project.

Questionnaires
Data files that contain information from the questionnaires and the ultrasound investigation have a code number. This code number, which is the same as the one in the person database, will only be used together with the national identification number in the event that links are to be made to other health registries or data is to be extracted for case-control studies. The questionnaire databases will be stored and checked at the Medical Birth Registry in Bergen. For content, see appendix 6,7, 8 and 9.
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Collaboration with Medical Birth Registry (MBR)
Data from the standard notification form to MBR will be included in the database for the Norwegian Mother and Child Cohort Study. The Norwegian Data Inspectorate has granted a concession for this. This is an important link, as it will prevent the project contacting the parents of children who have died at, or soon after birth, as well as allowing the identification of multiple births. Case control studies that are directed towards the parents of children who are born with congenital abnormalities or other specific pregnancy outcomes will also be possible.

Blood samples
The aliquoted blood samples will be frozen to -80^{o}C and stored in freezers at the Norwegian Institute of Public Health. Extracted DNA will be stored at -20^{o}C. A specially constructed data application will be used to locate the blood- and urine samples. These can only be removed with a concession from the Norwegian Data Inspectorate.

Database links
All links to databases other than MBR should be approved by the Norwegian Data Inspectorate.

Table 3
Flowchart for data collection

	What happens:		
Week/mth	**Hospital**	**MBR**	**Inst.of Public Health**
10-14	Before ultrasound		
	Receive names and addresses of pregnant women generally from referring GP.		
	A copy of list sent to MBR each week.		
	Send information by post to women	Send invitation by post to women with questionnaire 1, one questionnaire for the partner and consent form for both.	
		Receive consent form and questionnaires from participating women and partners	
17	**Ultrasound examination**		
	Women are asked if they will/will not participate		
	Blood- and urine samples taken		
		Receive copy of standard ultrasound form	Receive blood- and urine samples from women and partners
	Later in pregnancy		
18		Send newsletter from the study as a reminder for missing consent form and questionnaires	
22		Questionnaire 2 sent out	
30		Questionnaire 3 sent out	
		Reminder sent after 3 weeks	
	Birth		
	Blood samples from mother and umbilical cord after birth		
			Receive blood samples from mother and child
	Age 6 months		
6 mth		Send out questionnaire 4	
	Age 18 months		
18 mth		Send out questionnaire 5	
	Age 36 months		
36 mth		Send out questionnaire 6	

Pilot surveys

A small-scale trial survey was completed and the questionnaires and routines for taking and sending blood samples were tested. The overall experience was positive. Physicians working in primary health care collaborated in this study, but after discussion within the Norwegian Medical Association recruitment by this channel was abandoned. From summer 1999, recruitment has been as described above. At the end of 2004, approximately 50 000 women had been recruited. The overall participation rate is about 43% of all invited women..

In September 2000, a pilot study was started at the Hospital of Bærum with inclusion of the fathers. The reason for this is that occupational hazards, ill health and use of medications by the father may potentially cause mutations in reproductive cells, which can lead to illness in the child. Fathers were asked to complete a questionnaire and give a blood sample. The fathers are now included in all parts of the country. 80% of the fathers participates when women participates.

The project will not be able to require that all the participating hospitals follow exactly the same routines in recruitment, ultra sound examination and the taking of blood-and urine samples. Therefore, the project must be flexible and able to adapt to local conditions. Special support has, for example, been given to the midwives working at some hospitals and to laboratory staff at others.

The availability of economic resources has determined the study's progress. The project is now recruiting women from all parts of Norway, but a few of the larger hospitals are not yet participating.

Project Organization

Two teams engaged in perinatal epidemiological research started the project: One consisting of researchers at the Medical Birth Registry and The Department of Public Health and Primary Health Care at the University of Bergen; the other attached to the Section for Epidemiology, National Institute of Public Health, Oslo. After 01.01.02 both groups are parts of The Division of Epidemiology, Norwegian Institute of Public Health, where the study is anchored (Head: Camilla Stoltenberg). The Director of The Norwegian Institute of Public Health, Geir Stene- Larsen, heads the project technically, financially and administratively. Furthermore, the study has a leader team, consisting of 4 members from the division. The principal investigator is Per Magnus. A governmental committee evaluated the Norwegian Mother and Child study in spring 1998. General practitioners took the initiative for this. In Parliament, a large majority approved the study and the Ministry of Health and Social Affairs requested that the National Institute of Public Health carry out the study.

In order to carry out the data collection, the study has engaged full- or part time, laboratory technicians/midwives at the main participating hospitals. The period of employment is related to the length of time data will be collected in each region. The project has also employed staff in Bergen to manage the databases at MBR in Bergen.

International collaboration

The National Institute of Environment Health Sciences (NIEHS) in the US has signed a contract for 1,2 million dollars, giving them access to urine and blood samples from participating women. The purpose of their study is to examine the effect of environmental

toxins on childhood illness. The US National Institute of Health (NIH) is funding part of this project. Together with a group of researchers at The Colombia University, New York, a sub-study of Autism related disorders is planned (The ABC-Autism Birth Cohort). An amount of $ 13 mill from NINDS (National Institute of Neurological Disease and Stroke Health was granted in 2003 for this study. We collaborate with a group carrying out similar research in Denmark.

Funding

The Norwegian Ministry of Health granted the project a budget of NOK 1 million for 1998, 1999 and 2000, NOK 5 million for 2001 and 2002 and 6.5 million for 2003.

Through the Norwegian Research Council, funds have been set aside for research in functional genomic. 50 mill NOK over 5 years has been given to a technology platform for human biobanks and health studies, consisting of the Cohort of Norway (a cohort of 200 000 adults) and the Norwegian Mother and Child Cohort Study. In 2002, 4.5 million NOK was allocated to The Mother and Child Study.

The Confederation of Norwegian Business and Industry (NHO) has given NOK 1 million to the project through its occupational environment fund in 2000 and 2001. Private organizations and non-governmental research organizations are currently considering a number of applications.

Concessions from the Norwegian Data Inspectorate

In October 1996, the project was granted a concession by the Norwegian Data Inspectorate. This was renewed in September 2003. The Director at the Norwegian National Institute of Public Health is responsible for the registers. Together with the concession, the Norwegian Data Inspectorate also made recommendations relating to participant information and consent. The concession includes information from the Medical Birth Register and information and blood samples from the father. Meetings and correspondence between the Norwegian Data Inspectorate and the project have lead to, amongst other things, changes in the recruitment procedure.

The project involves collecting sensitive personal information. Much effort has already been, and will continue to be, expended on ensuring that third parties cannot link data from the project to a specific name. We require participants to state name, address and national identity number for two reasons. Firstly, we can communicate with participants during data collection and secondly, we can link data to external data sources, such as hospital records.

Data from the questionnaires, blood- and urine samples will be stored with a linkable code number. The code number and national identity number will only be linked when required in the course of data collection, and later when links to other databases are made. All the data made available to researchers (internal and external) will be stripped of personal identifiers; that is identification will be possible using the code number, but not directly to the person who has provided that information. As a general rule, the results from analyses of blood- and urine samples will be transferred back to the central database so that others can utilize them, but this can be evaluated in each individual case. When new data are being collected (in connection with a subproject requiring additional consent), consent shall also be obtained to transfer the data to the main database.

It should be particularly noted that the use of all blood- and urine samples would require approval from the Norwegian Data Inspectorate and the Regional Ethical Committee. All links to external data sources require a concession from the Norwegian Data Inspectorate.

The consent forms, letter inviting study participation and the brochure shall all give information regarding the scope of the study and consequences of participation. Newsletters will keep participants informed. Children will be informed personally about the study when they are 15 years. They will be asked to give active consent to continue in the study when they are 18.

Ethical considerations

"Contract" between participants and researchers
While rights and obligations have not been negotiated, the consent form in many ways resembles a contract, whereby participants agree to donate biological material and information about themselves. In exchange a guarantee is given that the researchers will use the material to study the causes of disease. This involves a fundamental trust on the part of the participants; it will be unethical on the part of the researchers not to utilize the material in the manner intended, or to use it for other purposes. New subprojects, which require active participation (completion of new questionnaires, clinical investigations, evaluation of exposure or new biological samples) beyond what is explicitly stated in the signed consent form, will require new consent. Subprojects that require collection of new data will need approval from Regional Ethics Committees and the Norwegian Data Inspectorate. Participants on subprojects must also give permission that data will be channeled into the main project.

Project value
The purpose of the project is to investigate the causes of disease. Knowledge about the causes of disease can lead to good interventions and further laboratory research which can reveal both the mechanisms that underlie disease processes, and lead to new treatment forms. It is also important to disprove false theories regarding the cause of disease and investigate which factors promote good health and absence of disease.

Potential Harm
No interventions will be undertaken in connection with the project, in the sense that conditions resulting in an exposure will not be wittingly modified in order to prevent disease. Participants will not receive the results of blood tests, or other information about themselves, that they are not already aware of. Participants can be recruited to projects on the basis of disease information (e.g. pelvic pain or incontinence) or pregnancy outcomes about which they are already informed (e.g. congenital abnormalities) or based on geographical location, child's date of birth etc. As a guiding principle, recruitment cannot occur on the basis of lifestyle; for example, smoking. If participants are to be recruited on the basis of findings from blood- and urine analysis, they must previously have given written consent stating that they are aware that they will be informed of the results of the blood- and urine analysis.

Some participants may find some questions offensive, others may find that the scope of the questionnaire is wider than they had expected. The drawing of blood samples can also be experienced as unpleasant.

Conflicts of interest between researchers
The study is a national resource that will be available to all bona fide researchers with a legitimate request for data. If different research groups have similar study aims and wish to use the same data, then flexibility will be encouraged and collaboration sought. The leader group's decision will be final in such matters.

Dissemination of results
Publications will be made according to further guidelines (see Appendix 4) Norwegian public health risk evaluations and advice will be given according to the existent guidelines.

Ethical evaluation
Ethical aspects of the project were discussed in a seminar in 1995. A transcript of contributions at this seminar is available on request (Norwegian only). Further, the project has been evaluated by the Regional Ethics Committees for medical research, Health Region II (REK II). This committee is kept informed of all modifications and has approved, for example, the trial project including fathers. From spring 1997 to spring 2000, the project had its own committee advising and making recommendations to the project's executive and working groups.

Appendix 1: Description of the person database

Introduction
The Norwegian Mother and Child Cohort Study uses an advanced database (tracking system) to register and follow the progress of participants through the various phases of the study. Oracle 7.3 has been used to create and access the database, and the screen formats have been developed using Oracle Forms 5.0 and Oracle Reports 3.0. The computers, in which the tracking system is installed, are connected to the local network at the Medical Birth Registry. To ensure the security of the database, MBR's standard password system is used.

The main menu of the tracking system shows the most important tasks in the Norwegian Mother and Child Cohort Study. The description in this appendix follows these tasks. All the tasks are important, but screen layouts are not shown.

The unit that is followed by the tracking system is the pregnancy, not the woman. This is because a woman can participate in the project during several pregnancies.

Registration of new ultrasound appointments
A list of all women receiving ultrasound appointments is compiled at each hospital. These lists are transferred to the person database at MBR either manually or in encrypted electronic form.

Electronic transfer is direct, while paper lists are keyed in manually. If a woman is already registered in the system, the information will be updated.

Data relating to pregnancies being registered for the first time are entered. This information includes name, address, date, and where the ultrasound will be carried out. If a woman is already registered, either for this or a previous pregnancy, notification will automatically appear on the screen and the date for the new ultrasound can be checked.

A pregnancy being registered for the first time in the tracking system is given the status ***INNREG (Registered).*** The only information about the pregnancy at this stage is the actual registration.

Registration of new ultrasound forms
The hospital sends a copy of the ultrasound form for all women who have agreed to participate in the study. Estimated delivery date and date of last menstruation are entered from this form. These variables are important so that future mailings from the project are sent out at the correct time. In addition a number of parameters are registered (*para*, *ab* and *gravida)* to ensure that individual pregnancies are kept completely separate. A variable noting the number of foetuses is used after the birth to ensure that the correct numbers of umbilical cord samples have been taken. Further information from the ultrasound examination is registered in a separate data file (see appendix 3).

Mailings, reminders and returned forms

Appendix table 1: Mailing overview

Mailing	Timepoint for mailing	Timepoint for reminder	Target group
Questionnaires 1 and 2 + consent form [1)]	approx. 3 weeks before u.s. exam.	at u.s. exam.	women with status INNREG (Registered)
Questionnaires 1 and 2 [1,2)]	after consent form received	3 weeks after mailing	women with status DELTAKER (Participant)
Questionnaire 3	in 30th or 31st week of pregnancy	3 weeks after mailing	women with status DELTAKER (Participant)
Questionnaire 4, one for each child	When the child is 6mths old	3 weeks after mailing [3)]	women with status DELTAKER(Participant)
Consent form reminders		monthly	women for whom samples or questionnaires have been received, but status INNREG (Registered)

1. Women attending ultrasound examination at hospitals where fathers are also being recruited will also receive an invitation for the father containing questionnaires and consent form.
2. Only sent to women who have entered the tracking system from other sources than through ultrasound clinics.
3. Does not apply to triplets who are treated individually.

When questionnaires 1 and 2 are sent out (line 1, table 1), status changes from INNREG (registered) to INVITERT (invited)

The return date for each kind of questionnaire is entered in the tracking system. Consent form reminders (last line, table 1) are only sent to women from whom samples, or one or more completed questionnaires, have been received.

Questionnaires that have been returned are scanned and interpreted by a high capacity Fujitsu scanner and the program Eyes and Hands in Windows NT. This program reads marked and numbered fields and also allows the operator to manually enter data that the program finds difficult to interpret. The questionnaires are scanned and stored as optical images, which are then displayed on the screen as they are read by the program. This allows the operator to add or modify codes when necessary. Each scanned questionnaire is allocated a code consisting of a pregnancy identification number and a number indicating the type of questionnaire. These code numbers are transferred to the person database to keep track of the questionnaires that have been scanned.

Returned consent forms
The consent form is an important item in the tracking system. When returned, the consent date is noted and the pregnancy is given the status DELTAKER (participant). Only pregnancies that have been allocated this status are considered to be part of the study, and will be followed up with further questionnaire mailings.

Change of status
It is apparent from the description so far, that the status of the pregnancy is decisive in allowing progress through the tracking system. As soon as a pregnancy is registered, it is given the status INNREG (registered). Thereafter, and for as long as it remains in the system, it will always have a status. The different types of status are shown in appendix table 2. When a woman, the Biobank or a hospital contacts the project indicating that the status of a pregnancy should be changed, a special command is used.

Appendix table 2 Overview of events which alter status in the tracking system

Status	Event resulting in change of status
INNREG (rgistered)	The pregnancy is registered in the tracking system.
INVITERT (invited)	First mailing to the expectant mother with status INNREG is sent out.
DELTAKER (participant)	Consent form returned.
ABORTERT (abortion)	Notification of abortion either from the woman herself or the hospital.
UTMELDT (withdrawn)	Notification of withdrawal from the study.
VIL SLETTES (wants to be deleted)	Notification of withdrawal and that all data is to be deleted.
SLETTET (deleted)	Notification from the Biobank that blood samples with status VIL SLETTES are deleted.
DØDFØDSEL(still birth)	Notification that the baby died (after 16th week) from MBR or woman herself.

Deleting a pregnancy

A pregnancy can be deleted if a woman having first agreed to participate and later wishes to withdraw and have all the data concerning her pregnancy deleted. The resulting status is VIL SLETTES (to be deleted), and the Biobank is requested to delete blood-and urine samples. Data already registered from questionnaires can also be deleted from the main data files. However, data that have been made anonymous and already given to researchers cannot be deleted.

On receipt of an acknowledgement from the Biobank that blood- and urine samples have been deleted, the pregnancy is again called up and the date for deletion of the blood sample entered. The status of the pregnancy is then automatically registered as SLETTET (deleted). At this stage, the only information remaining in the system is that the woman has participated in the study during the pregnancy. A letter is sent to the woman, stating that all the information about her has been deleted.

Data from the Biobank

Once a week, a computer generated encrypted list of newly registered blood-and urine samples is sent to the project from the Biobank. Data from this list are entered into the tracking system. This enables consent form reminders to be sent and compilations of pregnancies with complete sets of blood-and urine samples to be made.

Linking data from MBR and DSP

Before questionnaire 4 is sent out, data from the Medical Birth Registry (MBR) and National census data (DSP) are linked to the tracking system. This is to confirm the date of birth and ensure that questionnaire 4 is only sent to women having one or more living children in the registered pregnancy. In addition, information about multiple births (twins, triplets) enables an appropriate number of questionnaires to be sent.

Appendix 2: Biobank description

Biological samples will be collected from participants during the Norwegian Mother and Child study (Fig 1, page 23).

At ultrasound examination: 100 000 samples from pregnant women

At and after the birth:
100 000 umbilical cord samples from the child
100 000 maternal samples

Fathers are asked to participate and 80% participate.

A contract is signed with the National Institute of Environmental Health Sciences in the US to study the influence of environmental toxins for ill health in mother and child. For this study, special blood samples and urine sample will be taken from the mother at ultrasound examination. In December 2000, an international advisory group visited the National Institute of Public Health in Oslo and gave recommendations.

SAMPLING TECHNIQUE
All samples are taken at the hospital and sent to the Biobank at The Division for Epidemiology, Norwegian Institute of Public Health, Oslo for registration, processing and storage.

At ultrasound examination:
Samples taken from pregnant women, the K1-sample:
Tube 1: 7 ml EDTA vacutainer-tube with whole blood
Tube 2: 7 ml EDTA vacutainer-tube with whole blood
Tube 3: 7 ml EDTA vacutainer-tube with whole blood
Tube 4: 3 ml EDTA vacutainer-tube with whole blood
Tube 5: 8ml urine

Tube 2 is centrifuged in a standard centrifuge (not a chilled centrifuge) and 2.5 ml plasma is transferred to a 5 ml empty plastic tube.

To be sent to the Norwegian Institute of Public Health in Oslo:
Tube 1: 7 ml whole blood (EDTA)
Tube 2: 7 ml whole blood (EDTA
Tube 3: 4.5 ml blood (2.5 ml plasma removed)
Tube 4: 2.5 ml plasma
Tube 5: 3 ml whole blood (EDTA)
Tube 6: 8ml urine

Samples taken from fathers, the F-samples:
Tube 1: 7 ml whole blood (EDTA)
Tube 2: 7 ml whole blood (EDTA)
Tube 3: 2.5 ml plasma transferred from tube 2 (after centrifugation)

The tubes are sent to The Norwegian Institute of Public health

At birth
Cord blood, N:
Tube 1: 7 ml whole blood (EDTA)
Tube 2: 7 ml whole blood (EDTA)
Tubes are filled as much as possible using aspiration.

To be sent to The Institute of Health:
Tube 1: 7 ml whole blood (EDTA)
Tube 2: 7 ml whole blood (EDTA)

Maternal sample, K2:
Tube 1: 7 ml whole blood (EDTA)
Tube 2: 7 ml whole blood (EDTA)
Tube 3: 2.5 ml plasma in a plastic tube (transferred from tube 2 after centrifugation)

All samples are labeled with mother's date of birth and name, together with information on type of blood sample: K1, K2, N or F. This information is also labeled to a note stating where and when the sample was taken.

TRANSPORT
All samples are stored at 4°C until they are sent to the Norwegian Institute of Public Health.

If possible, blood- and urine samples should be sent the same day that they have been taken. The samples are sent as usual - not on ice or frozen. They can be sent as ordinary mail, by messenger or express delivery (over night). Information notes are sent together with the samples.

The samples are processed as soon as they arrive at the Norwegian Institute of Public Health. Exception: samples arriving on Saturdays will be stored at 4°C until Monday morning.

THE BIOBANK
A specially constructed data application (the Mother-Child-program) has been made for the Biobank. Its function is to keep track of where each individual sample is stored and ensure that samples cannot be mixed up. The program communicates with the software in a pipetting robot so that all the samples in the pipetting robot are correctly registered in the Mother-Child-program.

Registration of samples:
All in-coming samples are labeled with the woman's name and national identification number. The samples are registered in the specially constructed program using name, national identification number and the type of sample (K1, K2, N, F). In addition, the hospital's name and dates for sampling and arrival at the laboratory are registered. The contents of the sample (whole blood, plasma, urine) are also registered, together with comments when these apply, e.g. coagulated umbilical cord sample, too little blood in tube, etc.

On storage, each sample is given a sample code, and the national identity number is encrypted/protected. Access to the file, which allows the data to be deencrypted, will be strictly limited to authorized personnel.

Cord blood samples:
Umbilical cord blood will not be centrifuged at the hospitals. This will take place in the laboratory at the Norwegian Institute of Public Health, so that a plasma sample can also be obtained.

Subdividing the samples:
A robot-aided pipetting system is used to distribute full blood and plasma between 96-well microtiter and deep-well plates. All the plates are mechanically heat-sealed before they are frozen.

Whole blood: 1860 µl is transferred to a single well in a deep-well plate. A single plate will therefore store blood from up to 95 people and at least one control. Storage tp. -80° C. (fig 1). 3 ml whole blood (EDTA) is frozen without any processing

Plasma: A total of 1.8 ml plasma is divided on microtiter plates. 300 µl plasma from each person is stored, for example, in position 1B on six separate plates. Storage tp -80°C (fig 1).

Urine: Subdivided and frozen in deep well plates.

DNA-extraction:
In addition to whole blood and plasma, DNA is also stored.

DNA is extracted from the rest of the blood, using a DNA-extraction kit (Examples: PureGene, Gentra, FlexiGene). Approximately 150 – 700 mg DNA is obtained from each participant. The critical steps in the procedure, implying a risk that either samples or reagents are accidentally exchanged, are supported and controlled by the computer program. After the DNA is diluted leaving all samples with the same concentration, 100 ng/µl, a robotic system pipettes out 4 aliquots of 1,5 ml into deep-well plates. Storage tp -20° C. (fig 1).

DNA from umbilical cord blood is extracted using the same kit. The samples that have coagulated are frozen at -20°C. During thawing, clotted samples are crushed using a wooden spatula before the standard procedure is followed.

Storage:
Each sample has a unique laboratory code and a unique location.

The sample will have a designated location on a plate. The plates are previously marked with two labels with a unique code and are placed in a containing rack, which is also marked with unique codes. The freezers also have code numbers.

Samples from each participant are stored in at least two separate freezers, and there will always be an empty back-up freezer in case of breakdown. Alarms that have been installed on the freezers are activated if the temperature in the freezer rises 10 °C above the optimal.

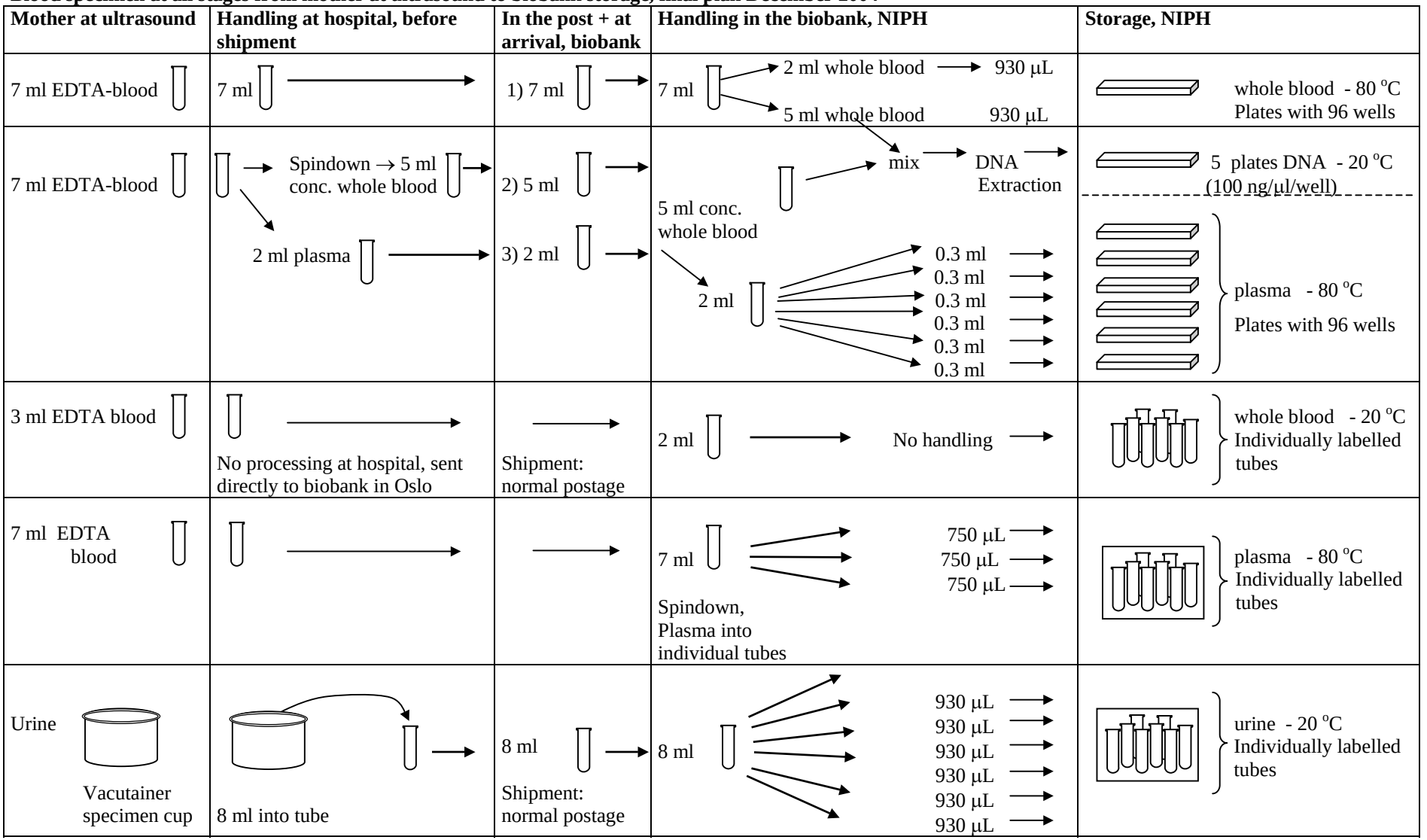
Retrieval:
When samples are to be retrieved, the starting point is a list of national identity numbers. This will be entered into our computer program, and the program will find the correct location for the sample. As all samples are stored in 96-well plates, they will be subject to thaw-freeze cycles each time a plate is retrieved. As certain analytic techniques require that a sample has only been subject to a limited number of thaw-freeze cycles, it is important to keep a record of how often plates have been thawed. Other samples from the same individual will always be available at another location, which has not been thawed. The seals on the plate are only broken for the samples in question, and these are rapidly resealed prior to refreezing.

Deleting samples:
In the event a woman wishes to leave the project and that all her data and biological material be removed, a delete function is found in the data program. All information about the woman and her samples is then deleted permanently from the files. Blood samples will remain in the freezer, but no data will be connected to these.

Communication with the Medical Birth Registry, Bergen:
Once a week a report is sent to MBR listing the samples that have been received at the laboratory. This information is encrypted and sent by electronic mail.

Information that a blood sample has not been taken is forwarded to MBR.

Figure 1, The Norwegian Mother and Child Study
Blood specimen at all stages from mother at ultrasound to biobank storage, final plan December 2004



Appendix 3: Description of the questionnaire database

Images of the questionnaires are scanned. These raw data files are stored. They make the starting point for coding variables which the scanner cannot manipulate directly, e.g. information about employment.

A database is constructed for each of the questionnaires and the ultrasound form. The databases can be linked using a serial number.

Quality controls will be carried out during and after the scanning process and before final storage.

A working group has been formed consisting of one person from The Norwegian Institute of Public Health in Oslo and one from MBR, who will make detailed instructions for quality control. The working group will ensure these instructions are carried out and that quality control is adequately documented. They will also suggest methods for checking the validity of the data.

Questionnaires having passed through the quality control will be stored in the databases. Each month, a copy of the databases at MBR in Bergen will be sent to The Division of Epidemiology in Oslo. Access to the data and agreements regarding analysis and publication, will be decided by the publications group.

The databases will be constructed and accessed using Oracle. Files intended for use by researchers for analysis, will be in SPSS format with a built-in codebook.

Appendix 4: Rules for access to data and publication

Aims
The aims are:

- to ensure rapid publication of all the important results of the study
- to ensure high scientific quality of the publications
- to ensure high availability of data for researchers working both internally and externally with the project group
- to set up procedures which enables the project's executive group to take responsibility for the professional content of the publications
- to maintain a complete list of all publications from the project
- to maintain a list over all sub-projects and ensure that access permission and obligations are complied with

Access to data
A major objective of the project is that data will be available to all researchers with relevant projects. Members of the project group and their collaborators will have free access to the questionnaire data and analysis files arising from linking the databases. These files will be available both at The Norwegian Institute of Public Health in Oslo, and MBR in Bergen.

Other researchers, nationally and internationally, will have access on request and following approval from the project's leader group. If necessary, contracts will be drawn up with external researchers. The leader group will evaluate new sub-projects. If several subprojects are found to be investigating the same or an overlapping topic, this will be resolved as far as possible by engaging in a direct dialogue with the parties involved.

In order for a request for data to become a recognized subproject, a short description that details the research question, variables to be investigated and the sample to be analyzed must be submitted. External researchers will be able to obtain an overview of the variables which are available by referring to the website.

For all subprojects, there will be conditions that either publication, or concrete plans for publication, will take place within three years after the data are made available. If this condition is not fulfilled, the data can be given to other researchers to study the same research questions. During the three years, the researchers will have exclusive rights to a particular research question. However, a high degree of specificity will be required in relation to the research questions.

If an external researcher requests a link between databases, which is not of general usefulness, and if much work is required with preparation and delivery of blood samples, a request for payment to cover costs will be made.

Publication
The leader group will coordinate research between The Norwegian Institute of Public Health and external researchers. The group is composed of two representatives from The Division of Epidemiology in Oslo and two from Bergen (MBR). Membership in the group is for a two-year period, but reappointment is possible.

Co-authors from The Norwegian Institute of Public Health both in Oslo and MBR will be required for major articles that describe associations between exposure and effect in the whole

sample. Suggestions for articles should be sent to the leader group who appoints an authors' committee with a named first author having overall responsibility for the data analysis and structure of the article as recommended in the Vancouver recommendations. The authors' committee is responsible for arranging working procedures and deciding the corresponding order of authors in the published manuscript. The leader group must approve all article drafts before they are submitted to a journal.

For articles that cannot be defined as major articles, it will be sufficient that only one institution is represented. In these cases, however, the leader group will also require a description of the research question, variables and selection, and approve the final draft of the manuscript.

Articles from subprojects will be required to cite at least one major publication from the project and will contain standardized paragraphs in the methods and materials section giving a brief account of the Norwegian Mother and Child study. This description will be available in English and Norwegian and will be written by the leader group. A copy of each article will be sent to the leader group prior to submission to a journal. The project's leadership will not have the authority to censure the professional content of the article, but will ensure that there are no factual errors about the project.

Appendix 5: Consent form

Consent form

for participation in the Norwegian Mother and Child Cohort Study

I have read the letter inviting participation and the information brochure about the Norwegian Mother and Child study and understand that the information I give will be treated strictly confidentially. I am aware that the project has been approved by the Regional Ethics Committee for medical research and by the Norwegian Data Inspectorate.

Participation in the Norwegian Mother and Child Cohort Study will entail the following:

- that I complete questionnaires, during and after pregnancy, about my own and my child's' health and living conditions
- that I give a blood sample and a urine sample, during pregnancy and one after the birth, and that a sample is also taken from the umbilical cord at birth
- that the blood samples from myself and my child will be stored and used in the future in research to study causes of diseases, including heredity. Laboratories in Norway and other countries will carry out this research following approval by the Regional Ethics Committee for medical research and the Norwegian Data Inspectorate that the blood samples can be used for this purpose.
- that the results from ultrasound examinations carried out during the pregnancy will be made available to the project
- that the blood sample which is taken from my child to test for PKU (phenylketonuria) may be made available to the project
- that no results (either concerning my own or my child's health) will be sent by the project to me
- that information about myself and the child can be obtained from other sources, for example the Medical Birth Registry and hospital records, following approval by the Norwegian Data Inspectorate
- that I can be asked to participate in further projects where I will be required to give additional information (or biological samples). Participation will be voluntary, and all such additional projects will satisfy conditions laid down by the Norwegian Data Inspectorate and the Regional Ethics Committee for medical research
- that information and blood samples will be stored indefinitely. This is a long-term study that will also investigate the reasons why diseases occur in adulthood. My child will be informed about the project when he/she is 15 years old, and consent requested from the child that he/she remains in the project when they are 18 years old
- that no information or biological samples will be made available to researchers before name and national identity number have been removed
- that participation is voluntary and that I can withdraw from the study at any time by writing to the Norwegian Mother and Child Cohort Study.

I have read the information above and agree to participate in the Norwegian Mother and Child Cohort Study.

Name: _____

National identity number *(11digits)*:_____

Date:_____ Signature:_____

My address on the invitation letter is wrong, the correct address is:

_____

Consent from the father

For participation in The Norwegian Mother and Child Cohort Study

I have read the invitation letter from The Norwegian Mother and Child Cohort Study and I understand that the information I give will be treated strictly confidentially. I am aware that the project has been recommended by The Regional Ethics Committee for medical research and approved by the Norwegian Data Inspectorate.

Participation in the Norwegian Mother and Child Cohort Study will entail the following:

- that I complete a questionnaire about my own health, life style and occupational environment
- that I give a blood sample at the time of my partner's ultrasound examination
- that the blood sample will be stored and used in the future for research purposes in order to study causes of disease, including heredity. Laboratories in Norway and other countries will carry out this research following approval by The Regional Ethics Committee for medical research and The Norwegian Data Inspectorate that the blood samples can be used for this purpose
- that no results will be sent by the project to me regarding my health including results from blood sample analysis
- that information about myself and the child can be obtained from other sources, for example the Medical Birth Registry and hospital records, following approval by the Norwegian Data Inspectorate
- that I can be asked to participate in further projects where I will be required to give additional information (or medical samples). Participation will be voluntary, and all such additional projects will satisfy conditions laid down by The Norwegian Data Inspectorate and The Regional Ethics Committee for medical research
- that information and blood samples will be stored indefinitely
- that no information or medical samples will be made available to researchers unless name and national identity number have been removed.
- that participation is voluntary and that I can withdraw from the study at any time by writing to The Norwegian Mother and Child study

Date:
Name:
Address:
National identity number:
Mother's name
Mother's National identity number:

Signature: